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This is report summarizes the final report of the subcontract, AFOSR F49620-91-C-0063 entitles "Effect of Halogenated Hydrocarbons on aquatic organisms" This research dealt with several experiments evaluating the sorption of halgenated hydrocarbons by different algae species. Three groups of algae species were tested. The sorption experiments were conducted under various conditions. With respect to changes in the growth medium composition, it was shown in this work that the silicon or nitrogen deficient medium in case of diatoms, or nitrogen or phosphate deficient medium in case of green algae or cyanobacterium could induce the higher sorption of halgenated hydrocarbons. Diatoms show better sorption than the other two groups. Low temperature had a positive influence on the sorption of halgenated hydrocarbons. In conclusion, when evaluating the sorption of halgenated hydrocarbons by algae, various algal species, incubation temperature as well as growth medium composition should be considered.

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Effects of Halogenated Hydrocarbons on Aquatic Organisms

Final Technical Report

to

Air Force Office of Scientific Research

Contract AFOSR: F49620-91-C-0063

August, 1995

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SUMMARY: This research dealt with several experiments evaluating the sorption of halgenated hydrocarbons by different algae species. Three groups of algae species were tested. The sorption experiments were conducted under various conditions. With respect to changes in the growth medium composition, it was shown in this work that the silicon or nitrogen deficient medium in case of diatoms, or nitrogen or phosphate deficient medium in case of green algae or cyanobacterium could induce the higher sorption of halgenated hydrocarbons. Diatoms show better sorption than the other two groups. Low temperature had a positive influence on the sorption of halgenated hydrocarbons. In conclusion, when evaluating the sorption of halgenated hydrocarbons by algae, various algal species, incubation temperature as well as growth medium composition should be considered.

INTRODUCTION

The presence of synthetic organic contaminants in the environment represents a threat to ecosystems, and many of these contaminants are toxic chemicals that pose both acute and chronic risks to human health. They cannot be easily broken down by subsurface microorganisms or filtered out by soils before they reach groundwater supplies. Especially troublesome are chlorinated hydrocarbons, classes of highly resistant, poorly degradable compounds. Many pesticides (e.g., DDT and lindane) are included in this class. Other dangerous chlorinated hydrocarbons in this class are the industrial organic solvents, such as trichloroethylene (TCE), carbon tetrachloride (CTC) and chloroform (CHF). Because of the hydrophobic character the chlorinated hydrocarbon solvents (CHSs) associate preferably with particles, the pathways of CHSs in the groundwater are strongly tied to the dynamics of particles. A large fraction of particles in natural waters consists of phytoplankton. Algae occupy an important position as the primary producer in aquatic ecosystems and they are the basis of many aquatic food chains. In addition to that, algae have high lipid content in their cells, especially the diatoms. Therefore, they are an important factor in controlling fate and transport of CHSs in ground water. While much attention is given to bioaccumulation of chlorinated hydrocarbon solvents in animals, such as oysters (Sericano, 1991), mussels (Doherty, 1993), fish and mammal (Chen, 1993), and the biodegradation of these solvents by bacteria (Murray, 1993), relatively few studies have focused on bioaccumulation of solvents in the algae, which play an important role in chlorinated hydrocarbon solvents exposure to zooplankton, fish, and mammals through the food chain.

Until recently, the research with chlorinated hydrocarbon solvents (CHSs) concentrated on two aspects: biodegradation of solvents by bacteria (anaerobic and aerobic); distribution and accumulation of CHSs in different levels of the food chain, including humans (Chen, 1993; Zylstra, 1989; Derek, 1988). However, very few papers are focused on the relationship between the chlorinated hydrocarbon solvents and algae that make up the base of the food chain in aquatic ecosystems.

OBJECTIVES OF THIS REPORT

The objectives of this study are to:

(1) Determine the uptake of chlorinated hydrocarbon solvents (CHSs) by diatoms, green algae and blue-green algae.

(2) Compare the differences in uptake of the CHSs between algal cultures incubated in complete medium, nitrogen deficient medium, silicon deficient medium and phosphorus deficient medium.

(3) Compare the differences in uptake of the CHSs between the algal cultures incubated at different temperature.

Algae are ubiquitous in aquatic ecosystems. They, as primary producers at the base of the food chain and as a major heterogeneous group of particles in natural waters, play a major role in the fate and transport of CHSs in aquatic ecosystem. If water quality is altered by CHSs from industrial, agricultural or municipal sources, algae are one of the first organisms to encounter the chemicals. Unlike the biodegradation occurred in bacteria, most researcher found that the organic chemicals sorbed by algae will accumulate in the algal cells. The CHSs sorbed by algal cells may be transferred to other trophic levels or to the sediment when the cells settle from the water.

The ability of the sorption of organic chemicals by algae depends on the nutrient level of the growth medium. Hannan and Patouillet (1972) found that the toxicity of hydrocarbons on algal species increased with decreasing nutrient concentrations.

Trichloroethylene reduced the growth of a marine diatom at low nitrate level of the growth medium(Fisher et. al., 1976). Kar and Sing (1979) found that the effects of various levels of nutrients such as potassium phosphate, calcium nitrate, and calcium chloride individually and in combination were studied for their effects on the toxicity of commonly used carbon tetrachloride, carbofuran and hexachlorocyclohexane when added to the growth medium of N_2-fixing cyanobacterium *Nostoc muscorum.* The toxicity of carbon tetrachloride can be reduced to some extent when the growth medium contains higher concentrations of the nutrients compared to the regular medium (Stratton 1986).

Different algal species reacted differently to pollutants. Research by Walsh (1977) indicated that species of the family Bacillariophyceae were the least sensitive when compared to those of the families Chlorophyceae and Rhodophyceae. species of Chlorophyceae accumulated more solvents than species of Bacillariophyceae. Species of the family Cyanophyceae responded differently to concentrations of the carbon tetrachloride. *Anabaena cylindrica* was more sensitive to low concentrations of the trichloroethylene than *Nostoc muscorum* (Scheuber and Wildman, 1977).

MATERIAL AND METHODS

STRAINS. Two kinds of diatoms (*Amphiprora hyalina* and *Navicula saprophila*) one kind of green algae (*Chloroccus* sp.) and one kind of cyanobacterium (*Anacystis nidulans*) are utilized in the experiment. These unicellular green algal strains and diatom strains were obtained from the Gulf of Mexico collection of Tadros (1985).

GROWTH MEDIA. The growth medium for all diatoms species was prepared according to Guillard and Ryther. (1975) It contains 18 g/L of artificial sea salt (Aquarium Salt) and enriched with nutrients to the composition "f/2" (pH 7.5). The nutrients had the following composition: $NaNO_3$ (Sodium nitrate), 0.17g/L(0.017g/L--1/10 Nitrate Medium); $NaH_2PO_4 \cdot 2H_2O$(Sod.phosphatemonobas.), 0.01g/L; Na_2CO_3 (Sodium carbonate), 0.05g/L; $Na_2SiO_3 \cdot 9H_2O$ (Sodium meta-silicate), 0.3g/L(0.03g/L--

1/10 Silicate Med.); $MgSO_4$ (Magnesium sulfate), 0.05g/L

To the above ingredients, 1 ml of Vitamin Mixture, 1 ml of Trace Elements Solution, and 1 ml of Ferric EDTA will be added. 1 ml of Vitamin Mixture containingThiamine, 0.1g/L; Biotin , 0.5g/L; Vitamin B_{12}, 0.5g/L. 1 ml of Trace elements containing H_3BO_3 (Boric acid), 0.568g/L; $ZnCl_2$ (Zinc chloride), 0.268g/L; $CuCl_2 \cdot 2H_2O$ (Cupric chloride), 0.252g/L; $Na_2MO_4 \cdot 2H_2O$ (Sodium molybdate), 0.252g/L; $CoCl_2 \cdot H_2O$ (Cobalt chloride), 0.042g/L; $FeSO_4$ (Ferrous sulfate), 1.36g/L; $MnCl_2 \cdot 4H_2O$ (Manganese chloride), 0.36g/L.

The growth medium for green species contains 9 g/L of artificial sea salt (Aquarium Salt). The nutrients had the following composition:$NaNO_3$ (Sodium nitrate), 0.15g/L(0.015g/L--1/10 Nitrate Medium); $NaH_2PO_4 \cdot 2H_2O$(Sod.phosphate monobas.), 0.01g/L(0.001g/L--1/10 Phosphate Med.); Na_2CO_3 (Sodium carbonate), 0.02g/L; $MgSO_4$ (Magnesium sulfate), 0.3g/L.To the above ingredients, 1 ml of Vitamin Mixture, 1 ml of Trace Elements Solution, and 1 ml of Ferric EDTA will be added.

The nutrients composition of growth medium for cyanobacterium are listed: $NaNO_3$ (Sodium nitrate), 1.5g/L(0.15g/L--1/10 Nitrate Medium); $K_2HPO_4 \cdot 3H_2O$ (Potas.phosphate dibasic), 0.38g/L(0.038g/L--1/10 Phosphate Med.); $CaCl_2$ (Calcium chloride), 0.025g/L; $Na_2SiO_3 \cdot 9H_2O$ (Sodium meta-silicate), 0.058g/L; $MgSO_4$ (Magnesium sulfate), 0.38g/L. To the above ingredients, 0.5 ml of Trace Elements Solution, and 0.5 ml of Ferric EDTA will be added.

INCUBATION CONDITIONS. A culture room was designed for the experiments. It was illuminated with fluorescent tubes with light intensities ranging from 200 to 500 foot candles (Ft.C.). The room temperature was kept at 30°C. The environmental incubator was also utilized, the temperature was set at 20°C, the light intensities were the same as culture room.

COMPLETE MEDIUM AND DEFICIENT MEDIA. Complete medium contains all the nutrients. Nitrogen deficient medium (for all species) contains 10% of the $NaNO_3$ of the complete medium. Silicon deficient medium (for diatoms only) contains 10% of the $Na_2SiO_3 \cdot 9H_2O$ of the complete medium. Phosphorus deficient medium contains 10% of the phosphate(for green algae and cyanobacterium) of the complete medium.

CHEMICALS. The chlorinated hydrocarbon solvents used in the experiments were: (1) Chloroform (Mallinckrodt) (2) Carbon Tetrachloride (Mallinckrodt) (3) Trichloroethylene (Mallinckrodt)

DRY WEIGHT DETERMINATION. Dry weight was determined using pre-dried and pre-weighted aluminum plate. The plate were dried at 30 °C for 24 hours before algal dry weight was determined.

HEADSPACE GAS CHROMATOGRAPHY METHOD. The principle of headspace gas chromatography (HSGC) technique is that the volatile materials can reach the phase equilibria in a close system under certain temperatures. Usually, the analyzed substance is in liquid phase, such as blood, food and waste water, or solid phase, while direct analysis being carried out on the equilibrium gaseous (vapor) phase. (Hachenberg, 1983) The most distinctive feature of the HSGC technique is that the concentration of analyzed substance contained in the gas phase is used to determine the nature and composition of the condensed phase with which it is in contact. By using the headspace G.C. in this research, we can directly measure the concentration of CHSs without doing any extraction. It makes the results much more accurate and precise. The advantage of this technique is the possibility of the determination of the volatile components of the samples containing high-boiling or nonvolatile components. The direct admission of such samples in the gas chromatography sometimes is impossible or unsuitable due to the insufficient sensitivity of the detectors or the possibility of undesirable contamination of the column. For example, direct injection of biological samples, such as blood and urine, will cause matrix inferences and contamination markedly shortens the GC column's life-time.

SAMPLE PREPARATION. During the experiment, algae were routinely grown on complete medium as a stock culture. The algae were inoculated in 3 different kinds of medium (complete medium, nitrogen deficient medium and silicon or phosphorus deficient medium) by adding 30ml algal stock culture to 200 ml medium. Algae were grown in different media at 30°C for 24 hr. Algal cultures were harvested and suspended at the same concentrations (percent transmittance = 30%). 10 ml such algal suspension was transferred to a GC sample vial (20 ml), and 2.5 µl solvent was introduced by microsyringe. The GC sample vial was sealed with 20-mm Teflon-lined rubber septum, then capped by 20-mm aluminum crimp cap. The contents of the GC vials were mixed by using a Rotary shaker(Thermolyne) in the culture room (30°C) or controlled environment chamber(Puffer hubbard) (20°C) with same light intensity (200 foot candles). Batch experiments were performed over 3, 5, 24 hr. to determine the concentration of the test solvent. The control experiments were set at same time without adding algae. All the tests were performed in duplicate.

GAS CHROMATOGRAPHY. The ability of algae to uptake CHSs, during treatment under various conditions was determined by measuring the disappearance of that compound by headspace gas chromatography. All samples were performed with a Hewlett Packard 5890 series II gas chromatography equipped with a flame ionization detector, a split/splitless capillary inlet system, a 25m x 0.32 mm i.d. fused-silica column coated with 0.17mm dimethyl polysiloxane, and a Hewlett Packard 19395A headspace sampler. The GC operating condition were, injection port temperature 200°C; detector temperature 250°C; column initial temperature/time 30°C, 2 min.; temperature/time ration 40°C/min.; column finial temperature/time 250°C, 1 min. The data were collected and handled by HP 3365 series II (version A.03.11) software system.

EXPERIMENTAL DESIGN AND COMPUTATION. To study the sorption of CHSs by algae, the randomized complete block design with factorial arrangement of four species, three chemicals, three media, three time effects, two temperatures was used. All samples replicated two times.

The sorption of CHSs by algae were calculated in following manner:

$$\text{SORPTION BY DRY WEIGHT} = \frac{K \times [\text{CONC. CONTROL[mM]} - \text{CONC. SAMPLE[mM]}]}{\text{DRY WEIGHT}}$$

$$k = \text{Mole. Weight of CHSs} \times \text{Volume of Sample}$$

The graphs were plotted by using sorption result versus time.

RESULT & DISCUSSION

The sorption of chlorinated hydrocarbon solvents (CHSs), chloroform, carbon tetrachloride, trichloroethylene by diatoms (*Amphiprora hyalina*, *Navicula saprophila*), green algae (*Chloroccus* sp.) and cyanobacterium (*Anacystis nidulans*) are shown in Table (1-8) and Figure (1-8).

MEDIUM EFFECT ON SORPTION OF CHSs BY ALGAE IN DIFFERENT TEMPERATURE

***Amphiprora hyalina* 20°C**

Figure 2 (a) shows the sorption of chloroform by *A. hyalina* in complete medium, growth medium deficient in sodium-nitrate and growth medium deficient in sodium-silicate at 20°C. Algae incubated in the two deficient media show better sorption of chloroform than algae incubated in complete medium. On the other hand, it also shows that the growth medium deficient in sodium-silicate induces the greatest sorption of chloroform by *A. hyalina*. Similar patterns of results were also obtained in the sorption of carbon tetrachloride and trichloroethylene by *A. hyalina* at 20°C (Figure 2 (b), (c)). However, in Figure 2 (b), a slightly different result was found. The highest sorbed values of carbon tetrachloride were obtained in the algae cultured in the growth medium deficient in sodium-nitrate instead of growth medium deficient in sodium-silicate.

***Amphiprora hyalina* 30°C**

Figure 3 (a) shows the sorption of chloroform by *A. hyalina* in complete medium, growth medium deficient in sodium-nitrate and growth medium deficient in sodium-silicate at 30°C. Algae incubated in the two deficient media show better sorption of chloroform than algae incubated in complete medium. Similar patterns of results were

also obtained in the sorption of carbon tetrachloride and trichloroethylene by *N. Saprophila* at 30°C (Figure 3 (b), (c)). There is no fixed pattern for the relationship between the sorptions of CHSs by algae grown in two deficient media. Only in Figure 3 (b), the algae in growth medium deficient in sodium-silicate show better sorption than algae in the growth medium deficient in sodium-nitrate in all three batches of samples.

***Navicula saprophila* 20°C**

Figure 4 (a) shows the sorption of chloroform by *N. Saprophila* in complete medium, growth medium deficient in sodium-nitrate and growth medium deficient in sodium-silicate at 20°C. Algae incubated in the two deficient media show better sorption of chloroform than algae incubated in complete medium. On the other hand, it also shows that the growth medium deficient in sodium-silicate induces the greatest sorption of chloroform by *N. Saprophila.* Similar patterns of results were also obtained in the sorption of carbon tetrachloride and trichloroethylene by *N. Saprophila* at 20°C (Figure 4 (b), (c)).

***Navicula saprophila* 30°C**

Figure 5 (a) shows the sorption of chloroform by *N. Saprophila* in complete medium, growth medium deficient in sodium-nitrate and growth medium deficient in sodium-silicate at 30°C. Algae incubated in the two deficient media show better sorption of chloroform than algae incubated in complete medium. On the other hand, it also shows that the growth medium deficient in sodium-silicate induces the greatest sorption of chloroform by *N. Saprophila.* Similar patterns of results were also obtained in the sorption of carbon tetrachloride and trichloroethylene by *N. Saprophila* at 30°C (Figure 5 (b), (c)). However, in Figure 5 (b), a slightly different result was found. Only in 5 hour's sample, algae in growth medium deficient in sodium-silicate have better sorption of carbon tetrachloride than algae in the medium deficient in sodium-nitrate.

***Chloroccus* sp. 20°C**

Figure 6 (a) shows the sorption of chloroform by *Chloroccus* sp. in complete medium, growth medium deficient in sodium-nitrate and growth medium deficient in sodium-phosphate at 20°C. Algae incubated in the two deficient media show better sorption of chloroform than algae incubated in complete medium. On the other hand, it also shows that the growth medium deficient in sodium-phosphate induces the greatest sorption of chloroform by *Chloroccus* sp. Similar patterns of results were also obtained in the sorption of carbon tetrachloride and trichloroethylene by *Chloroccus* sp. at 20°C (Figure 6 (b), (c)).

***Chloroccus* sp. 30°C**

Figure 7 (a) shows the sorption of chloroform by *Chloroccus* sp. in complete medium, growth medium deficient in sodium-nitrate and growth medium deficient in sodium-phosphate at 30°C. Algae incubated in the two deficient media show better sorption of chloroform than algae incubated in complete medium. On the other hand, it

also shows that the growth medium deficient in sodium-phosphate induces the greatest sorption of chloroform by *Chloroccus* sp. Similar patterns of results were also obtained in the sorption of carbon tetrachloride and trichloroethylene by *Chloroccus* sp. at 30°C (Figure 7 (b), (c)).

***Anacystis nidulans* 20°C**

Figure 8 (a) shows the sorption of chloroform by *A. nidulans* in complete medium, growth medium deficient in sodium-nitrate and growth medium deficient in potassium-phosphate at 20°C. Algae incubated in the two deficient media show better sorption of chloroform than algae incubated in complete medium. On the other hand, it also shows that the growth medium deficient in potassium-phosphate induces the greatest sorption of chloroform by *A. nidulans*. Figure 8 (b) shows that algae incubated in the two deficient media show better sorption of carbon tetrachloride than algae incubated in complete medium. Figure 8 (c) shows that only algae incubated in growth medium deficient in potassium-phosphate shows better sorption of trichloroethylene than algae incubated in complete medium.

***Anacystis nidulans* 30°C**

Figure 9 (b) shows the sorption of carbon tetrachloride by *A. nidulans* in complete medium, growth medium deficient in sodium-nitrate and growth medium deficient in potassium-phosphate at 30°C. Algae incubated in the two deficient media shows better sorption of carbon tetrachloride than algae incubated in complete medium. On the other hand, it also shows that the growth medium deficient in potassium-phosphate induces the greatest sorption of carbon tetrachloride by *A. nidulans*. Similar patterns of results were also obtained in the sorption of chloroform and trichloroethylene by *A. nidulans* at 30°C (Figure 9 (a), (c)). However, in Figure 9 (a), a slightly different result was found. Only 24 hour sample shows that the algae in growth medium deficient in potassium-phosphate have better sorption of chloroform that in growth medium deficient in sodium-nitrate.

Previous researches (Pandiripally, 1994, Tadros & Johansen 1988) indicate that these chlorinated hydrocarbon solvents (CHSs) are generally toxic to phytophlankton. However, these researches also indicated that some species such as *Amphiprora hyalina* were resistant to the CHSs. The results we obtained indicate that when algae were grown under the nutrient deficient medium, they could sorb larger amount of CHSs from the aquatic environment than it does under the complete medium. This phenomenon could be explained by the passive equilibrium partitioning between a lipid and an aqueous phase, and by the accumulation of the lipid content of the algae under different nutrient condition.

In Herman's (1991), he describes the sorption of organic chemicals, in this case CHSs, as a lipid solubilization process. It suggested that CHSs molecules could be expected to partition into all compartments of the algal cell and reach thermodynamic equilibrium with lipid pools of the cell as a whole.

As mentioned before, both nitrogen-deficient medium and silicon-deficient

medium can cause the lipid accumulation in algae (Ben-Amotz, 1985; Shifrin 1981; Roessler, 1990). Tadros & Johansen (1988) found that the nutrient deficiency can cause the lipid accumulation in both *N. saprophila* and *A. hyalina*, and both of the algal strains show greater lipid accumulation in silicon-deficient medium than nitrogen-deficient medium. So, the nutrient deficient medium can induce the lipid accumulation in algae, which could make the algae higher capability of CHSs sorption from the aquatic environment.

Different algal species responded differently to organic chemicals. Research by Walsh (1977) indicated that *Chlorococcum* sp. accumulated more solvents than *Thalassiosira pseudonana.* Species of the family Cyanophyceae responded differently to concentrations of the carbon tetrachloride. *Anabaena cylindrica* was more sensitive to low concentrations of the trichloroethylene than *Nostoc muscorum* (Scheuber and Wildman, 1977). Our result also shows that there is a signification difference between the *Amphiprora hyalina* and *Navicula saprophila,* and *A. hyalina* generally shows a better sorption of CHSs than *N. saprophila*(appendix B). This might be explained by the different lipid content of the two algal species. It is also found that there is a significant difference between the diatoms and green algae, and between the diatoms and cyanobacterium. This might be due to the higher lipid content in the diatoms than in the green algae and cyanobacterium.

As mention before, the temperature effect on the sorption of organic chemical by phytoplankton is still largely undetermined. Few papers have been published on this subject. Different kinds of relationships between the temperature and the sorption of organic chemicals have been found (Szecsody, 1991; Horzempa, 1983; Schrap, 1991). The temperature can affect both the algal cultures and the CHSs, it can influence the algal CHSs sorption. Our data show that algae generally have better sorption of CHSs in 20^0C than in 30^0C. Further investigation are needed to explain this result.

CONCLUSIONS

Based on the results we got, we found that the nutrient deficient medium can induce the lipid accumulation in algae, which could make the algae higher capability of CHSs sorption form the aquatic environment; there is significant different of the CHSs sorption among the algal species; temperature is an important factor of the CHSs sorption by algae.

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Table 1: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *A. hyalina* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 20°C.

A. hyalina, Chloroform,20°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	55.25	94.09	87.79
5 HR	70.77	106.94	125.23
24 HR	71.12	122.10	128.40

A. hyalina, Carbon Tetrachloride,20°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	12.66	48.12	36.18
5 HR	17.25	44.68	34.14
24 HR	17.98	49.52	45.86

A. hyalina, Trichloroethylene,20°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	47.25	77.01	107.51
5 HR	53.75	79.00	105.33
24 HR	159.69	267.16	290.17

COMP. MED.= COMPLETE MEDIUM

N-DEF. MED = NITROGEN DEFICIENT MEDIUM

Si-DEF. MED = SILICON DEFICIENT MEDIUM

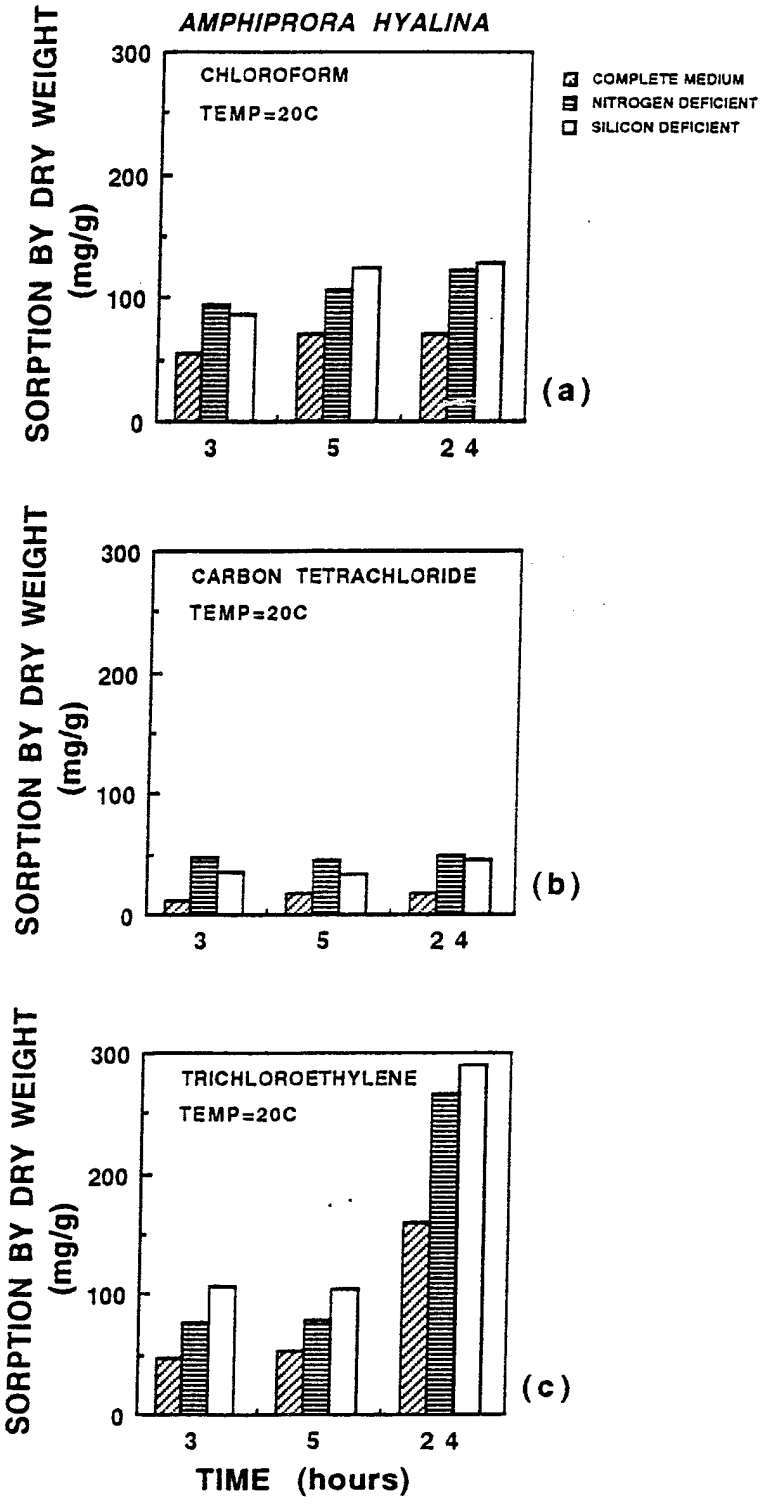

Figure 1: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *A. hyalina* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 20°C.

Table 2: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *A. hyalina* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 30°C.

A. hyalina, Chloroform,30°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	44.71	86.57	63.28
5 HR	63.65	111.55	85.16
24 HR	70.48	109.24	116.27

A. hyalina, Carbon Tetrachloride,30°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	13.94	53.12	70.66
5 HR	32.84	63.12	79.49
24 HR	33.21	84.67	100.89

A. hyalina, Trichloroethylene,30°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	24.29	45.77	47.01
5 HR	25.12	56.05	57.45
24 HR	30.09	75.53	75.44

COMP. MED.= COMPLETE MEDIUM

N-DEF. MED = NITROGEN DEFICIENT MEDIUM

Si-DEF. MED = SILICON DEFICIENT MEDIUM

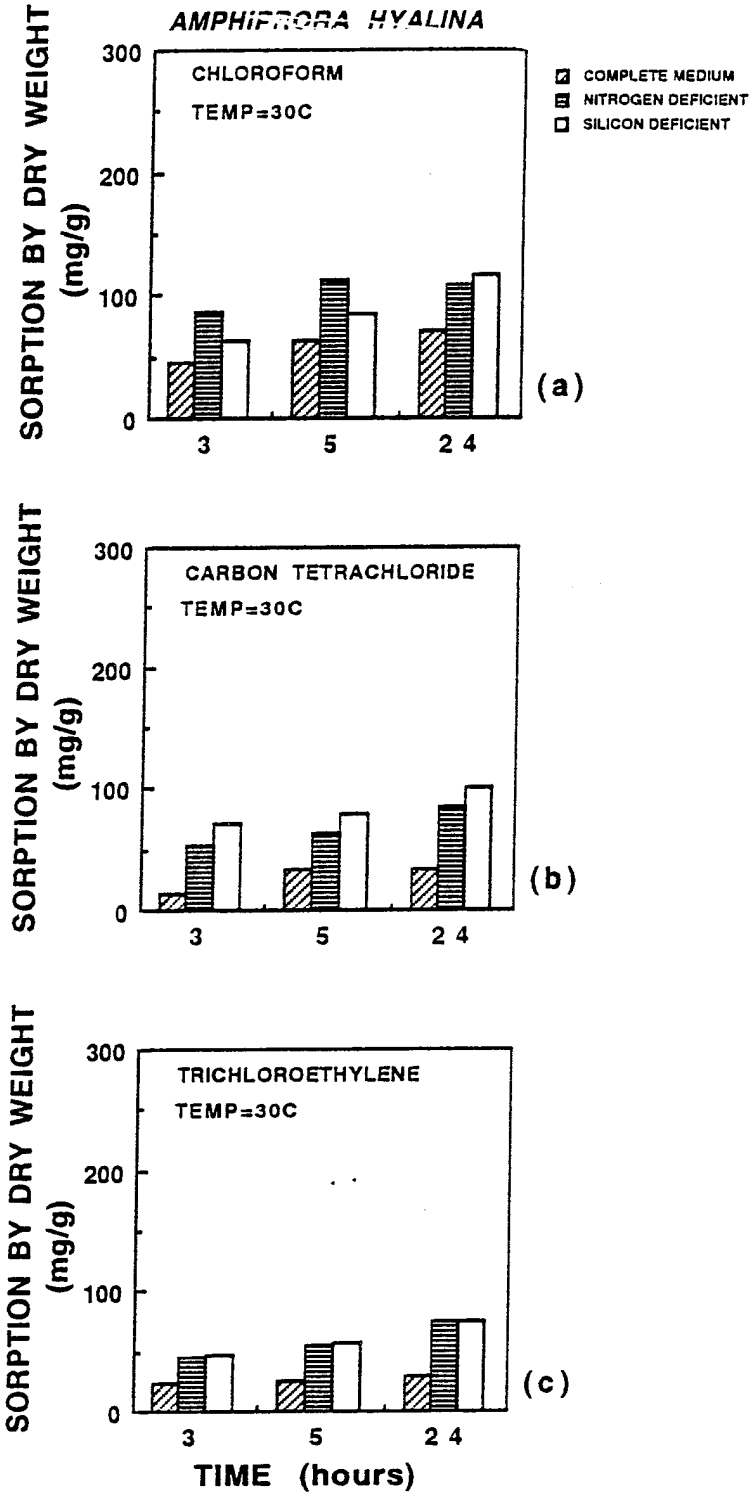

Figure 2: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *A. hyalina* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 30°C.

Table 3: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *N. saprophila* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dryweight) in three different media at 20°C.

N. saprophila, Chloroform,20°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	34.93	52.67	61.06
5 HR	30.94	53.33	59.54
24 HR	41.61	60.28	64.70

N. saprophila, Carbon Tetrachloride,20°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	13.37	25.66	36.79
5 HR	13.37	27.73	40.12
24 HR	18.90	34.96	43.64

N. saprophila, Trichloroethylene,20°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	14.06	32.36	47.98
5 HR	23.17	49.43	61.35
24 HR	26.69	55.75	66.36

COMP. MED.= COMPLETE MEDIUM

N-DEF. MED = NITROGEN DEFICIENT MEDIUM

Si-DEF. MED = SILICON DEFICIENT MEDIUM

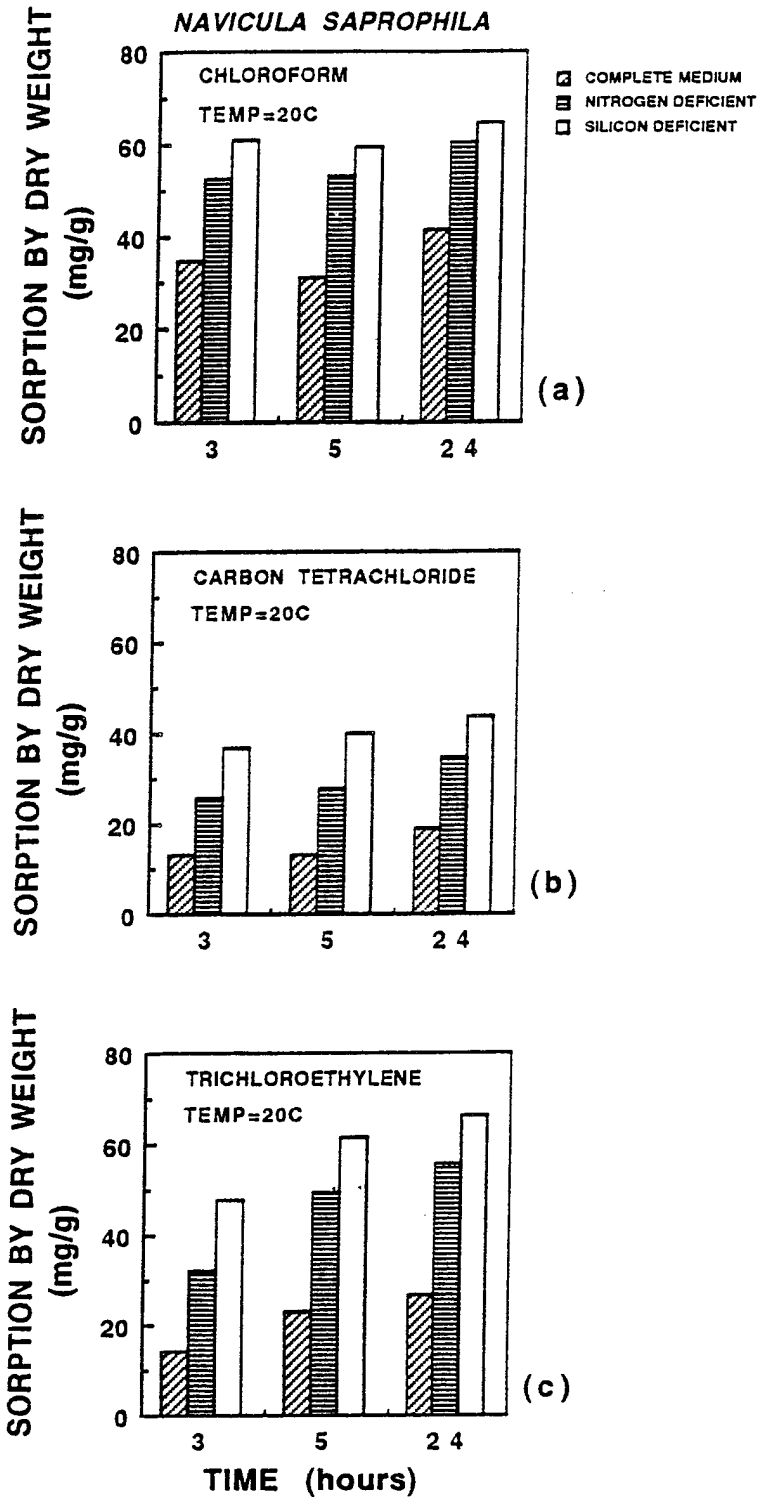

Figure 3: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *N. saprophila* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dryweight) in three different media at 20°C.

Table 4: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *N. saprophila* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 30°C.

N. saprophila, Chloroform,30°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	17.26	27.27	29.01
5 HR	23.35	26.73	33.57
24 HR	23.85	43.04	50.73

N. saprophila, Carbon Tetrachloride,30°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	18.26	26.51	26.22
5 HR	19.67	26.52	39.79
24 HR	29.32	46.67	46.58

N. saprophila, Trichloroethylene,30°C

TIME	COMP. MED.	N-DEF. MED.	Si-DEF. MED.
3 HR	14.06	32.36	47.98
5 HR	23.17	49.43	61.35
24 HR	26.69	55.75	66.36

COMP. MED.= COMPLETE MEDIUM

N-DEF. MED = NITROGEN DEFICIENT MEDIUM

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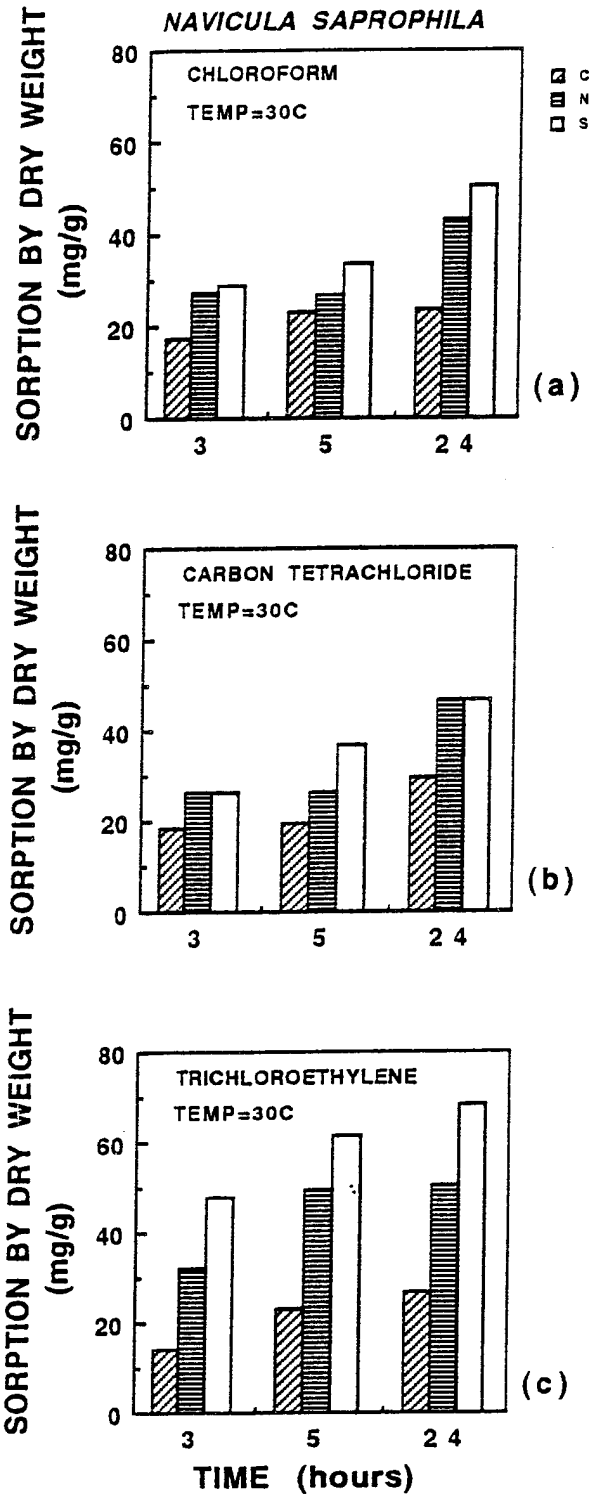

Figure 4: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *N. saprophila* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 30°C.

Table 5: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *Chloroccus* sp. expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 20°C.

Chloroccus sp., Chloroform, 20°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	12.34	22.70	41.04
5 HR	26.04	29.59	46.91
24 HR	38.03	49.45	64.50

Chloroccus sp., Carbon Tetrachloride, 20°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	25.61	24.55	40.29
5 HR	25.17	36.56	52.89
24 HR	28.04	29.77	62.45

Chloroccus sp., Trichloroethylene, 20°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	0.38	2.23	7.31
5 HR	6.60	15.62	42.59
24 HR	23.76	31.68	86.47

COMP. MED.= COMPLETE MEDIUM

N-DEF. MED = NITROGEN DEFICIENT MEDIUM

P-DEF. MED = PHOSPHORUS DEFICIENT MEDIUM

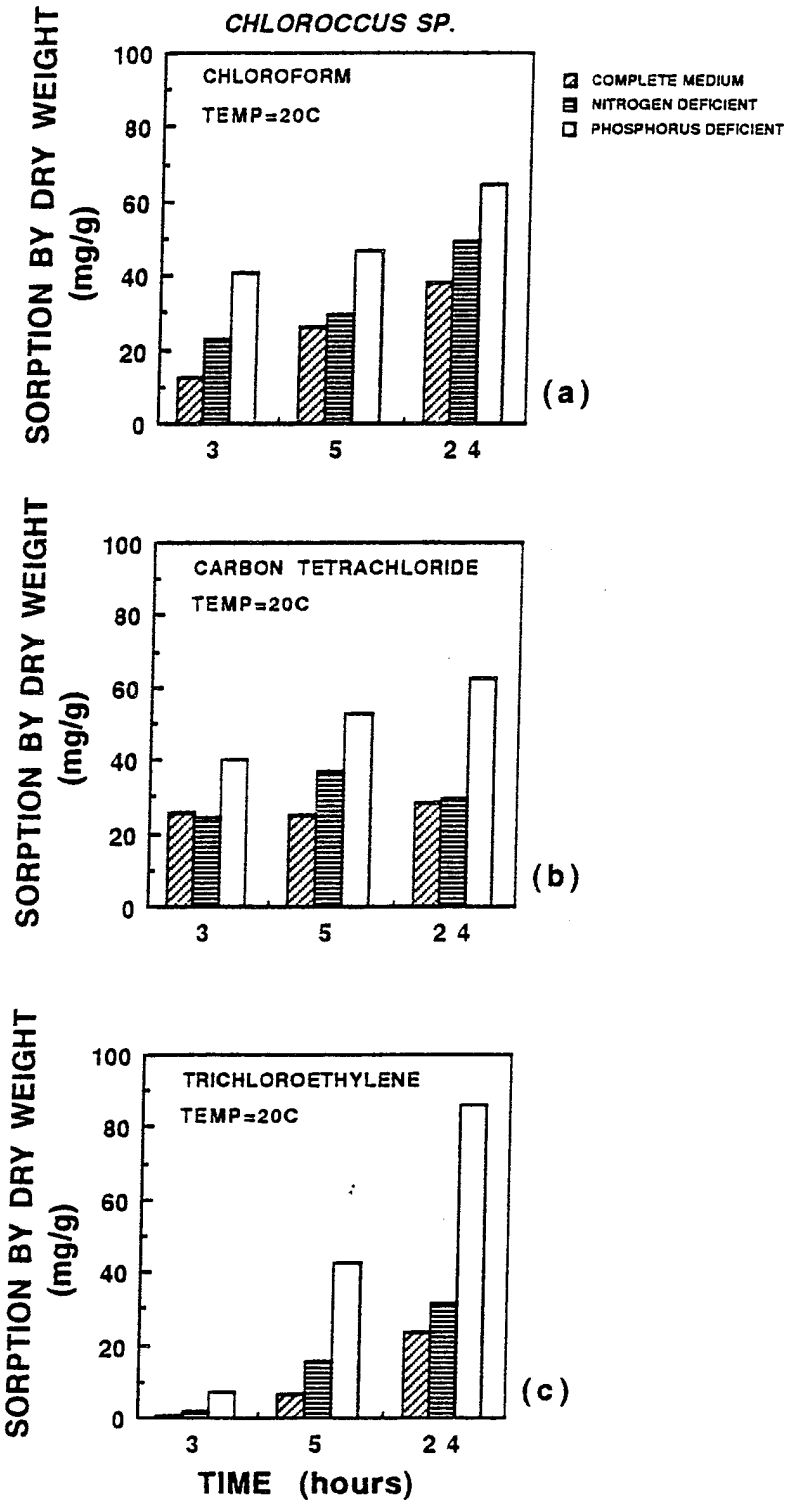

Figure 5: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *Chloroccus* sp. expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 20°C.

Table 6: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *Chloroccus* sp. expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dryweight) in three different media at 30°C.

Chloroccus sp., Chloroform, 30°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	3.77	15.00	32.05
5 HR	4.11	12.97	47.69
24 HR	17.65	26.55	57.66

Chloroccus sp., Carbon Tetrachloride, 30°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	4.42	7.31	28.2
5 HR	5.30	11.49	25.18
24 HR	5.74	12.54	28.2

Chloroccus sp., Trichloroethylene, 30°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	13.58	17.40	22.80
5 HR	15.27	54.88	79.16
24 HR	18.67	50.25	82.17

COMP. MED.= COMPLETE MEDIUM

N-DEF. MED = NITROGEN DEFICIENT MEDIUM

P-DEF. MED = PHOSPHORUS DEFICIENT MEDIUM

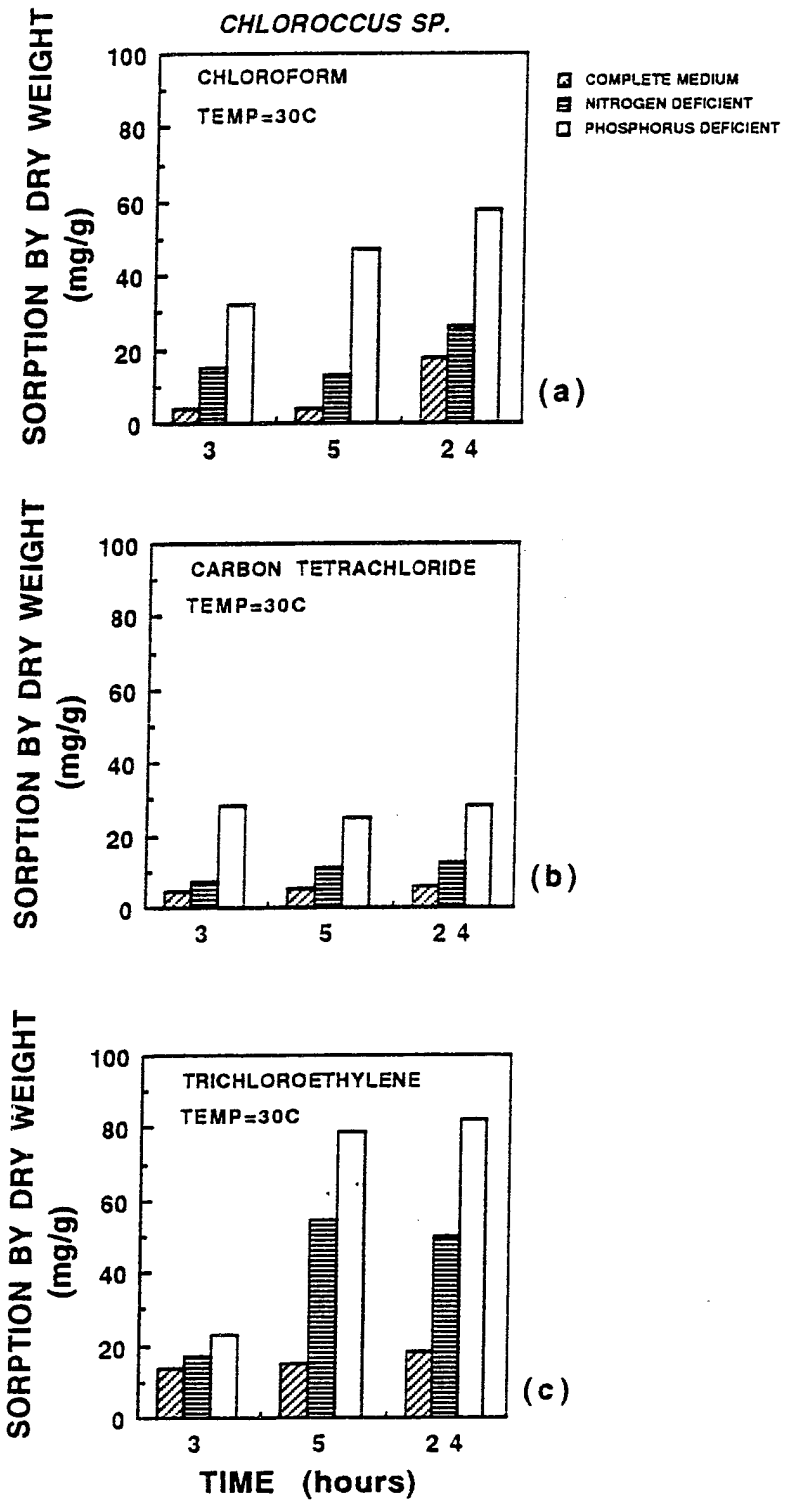

Figure 6: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *Chloroccus* sp. expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dryweight) in three different media at 30°C.

Table 7: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *A. nidulans* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 20°C.

A. nidulans, Chloroform, 20°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	20.48	78.28	110.41
5 HR	19.31	84.81	114.55
24 HR	46.82	86.76	133.18

A. nidulans, Carbon Tetrachloride, 20°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	1.51	10.09	12.45
5 HR	9.80	16.81	25.78
24 HR	8.29	57.16	44.46

A. nidulans, Trichloroethylene, 20°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	17.39	0.72	7.59
5 HR	14.81	10.77	81.26
24 HR	26.41	20.10	98.73

COMP. MED.= COMPLETE MEDIUM

N-DEF. MED = NITROGEN DEFICIENT MEDIUM

P-DEF. MED = PHOSPHORUS DEFICIENT MEDIUM

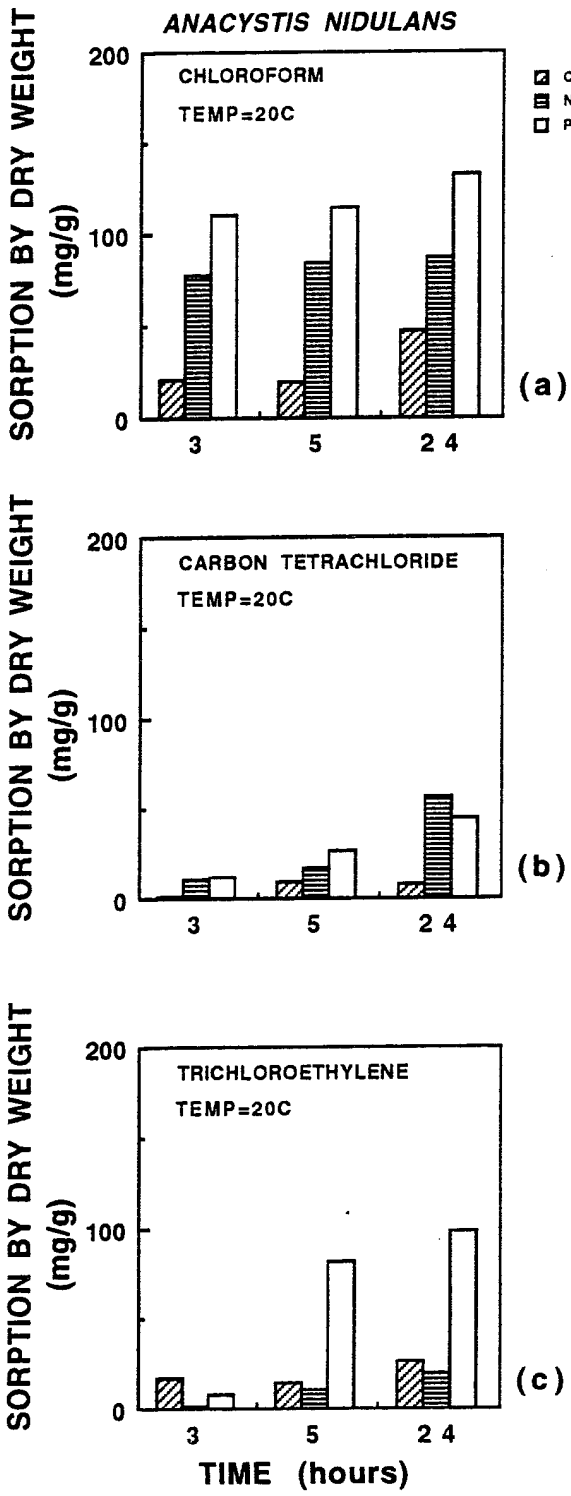

Figure 7: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *A. nidulans* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 20°C.

Table 8: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *A. nidulans* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 30°C.

A. nidulans, Chloroform, 30°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	3.51	46.32	20.70
5 HR	5.59	44.36	32.43
24 HR	37.75	51.01	86.95

A. nidulans, Carbon Tetrachloride, 30°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	2.82	10.09	46.23
5 HR	5.28	12.61	49.79
24 HR	6.79	48.75	75.13

A. nidulans, Trichloroethylene, 30°C

TIME	COMP. MED.	N-DEF. MED.	P-DEF. MED.
3 HR	4.51	3.59	7.59
5 HR	9.02	46.67	121.90
24 HR	82.44	118.47	213.41

COMP. MED.= COMPLETE MEDIUM

N-DEF. MED = NITROGEN DEFICIENT MEDIUM

P-DEF. MED = PHOSPHORUS DEFICIENT MEDIUM

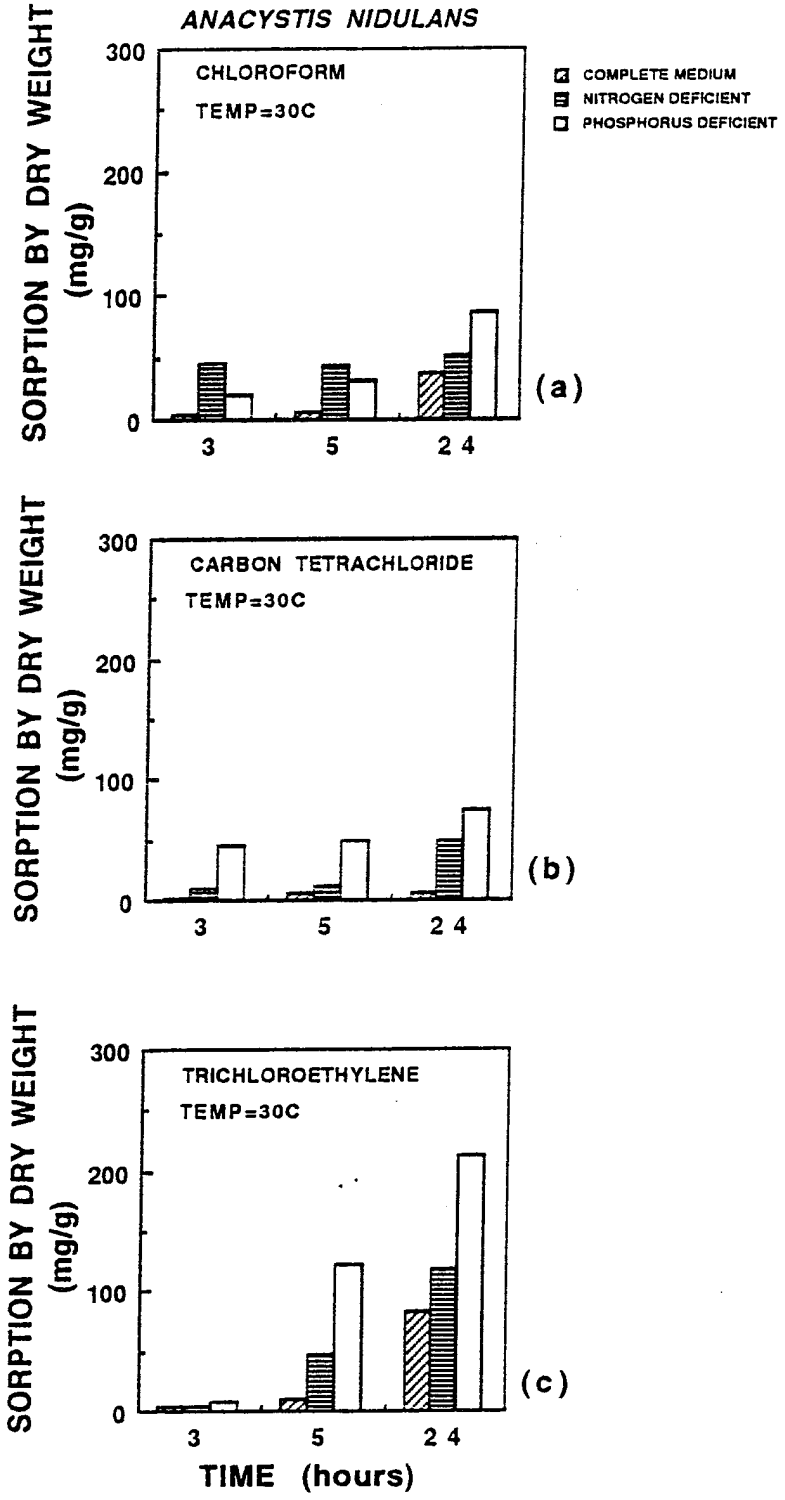

Figure 8: Variation with time of sorption of chloroform, carbon tetrachloride and trichloroethylene by *A. nidulans* expressed as the amount (mg) of CHSs uptaked by 1 g of algal biomass (Dry weight) in three different media at 30^{0}C.

SCIENTIFIC PERSONNEL SUPPORTED:

The following personnel have been involved in this project:
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Xin Wang, M.S.
Anthony E. Sharpe, M.S.
Greta James, B.S.
Chenxi Tang, M.S.

PRODUCTS OF RESEARCH:

1. Vinod K Pandiripally, 1994, Effect of solvents and nutrient stree on mixed algal species.
2. Xin Wang, 1995, Sorption of chlorinated hydrocarbon solvents by algae.
3. Tadros, M. G. etc. 1995, Differential response of marine diatoms to solvents, Bulletin of Environ. Contamin. Toxic. 54:924-929
4. Tadros, M. G. etc. 1994, Differential response of green algal species to solvents, Bulletin of Environ. Contamin. Toxic. 52:333-337